Solving the AAV Scalability Challenge

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See how our team worked with Sartorius to demonstrate consistent productivity, quality, and potency from 250 mL to 200 L scales.

Gene to GMP AAV manufacturing partner, Ascend, made a presentation at the 28th European society of Animal cell technology meeting, about leveraging technology to build a flexible and reliable platform. Working with Sartorius, the team has demonstrated consistent productivity (viral genome and capsid titers), quality (packaged host-cell and plasmid DNA, percent full capsids), and potency from 250 mL to 200 L scales with both the proprietary split plasmid EpyQ™ AAV production system or traditional triple transfection. A review of the challenges and results follows.

Introduction

Adeno-associated viral (AAV) vectors have a clearly demonstrated ability to successfully deliver gene therapies. Minimal immunogenicity and significantly limited ability to replicate offer important advantages, as does the varied tropism of an ever-expanding array of AAV serotypes.

AAV discovery is still in the early stages for the pharmaceutical industry. Cost-effective and seamlessly scalable AAV manufacturing processes are still needed, as are industry standards. Understanding this, Ascend Advanced therapies has built a flexible and enabling manufacturing platform, including the well-characterized portfolio of lab- to production-scale bioreactors from Sartorius Stedim Biotech (Sartorius). From 250 mL to 200 L scales, positive results have been demonstrated with both the EpyQ AAV production system and customer-developed triple transfection processes.

Overcoming AAV process scaling challenges

Transient transfection processes for the manufacture of AAV vectors require control of numerous starting material attributes and process parameters. Design of experiment (DoE) approaches performed in robust scale-down devices such as small-scale bioreactors for upstream process development, allow product specific optimization of modular platform processes at speed and with reasonable development costs.

Many process parameters are scale-dependent, making it difficult to constantly maintain multiple parameters (like mixing time, power input, impeller tip speed, and oxygen transfer rate (OTR)) simultaneously. Different cell seed-train volumes can also impact cellular performance, affecting product yield and quality. Indeed, many critical quality attributes (CQAs) related to product impurities including

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full/empty capsid ratios and residual plasmid and host-cell DNA (HCD) packaging levels are strongly determined by the specific biological starting materials used and the design of the upstream process.

Critical steps to effectively scale modular platform processes include:

STEP 1 Employ lab- to commercial-scale bioreactors from a single vendor for which vessel height-to-diameter ratios and impeller, baffle, and sparger designs support similar process conditions at different scales.

STEP 2 Choose optimal starting materials.

STEP 3 Immediate development of a platform process that targets comparable quality and productivity across all scales.

STEP 4 Use robust, fit-for-purpose analytics for in-depth product and process characterization.

With this strategy, consistent performance in terms of productivity, quality, and potency from the lab (250 mL) to commercial (200 L) scales – an 800-fold scaling factor – can be achieved.

Flexible & consistent performance across scales

Along with the proprietary split plasmid EpyQ AAV production system, the full portfolio enables processes that balance quality attributes with yield for multiple serotypes. Results are also positive using traditional triple transfection processes. In house purification protocols were established to fit various scales and application needs, considering material quality, and offering optional polishing steps for impurity reduction and removal of empty capsids. An unmatched range of owned and sourced analytics (> 50 assays) inform early process and formulation development, regulatory discussions, and product release.

To ensure consistent results as processes scale-up, Ascend employs a range of extensively characterized bioreactors from Sartorius for lab through commercial production. The current bioreactor platform includes Ambr® 15 and Ambr® 250 Modular systems and 2L, 5L, 50L, and 200L Biostat STR® bioreactors. In developing these systems, Sartorius has taken into consideration factors such as the gassing rate, liquid volume, and stirring speed, specific power input, and mixing time. Complete bioreactor characterization of many process parameters is achieved using Sartorius' BioPAT® Process Insights solution.

To clearly demonstrate comparable yield and quality across scales, a 3kb AAV9 vector containing the gene for secreted embryonic alkaline phosphatase (SEAP) reporter protein was produced in Ambr 250 and Biostat STR 2L, 5L, 50L, and 200L bioreactors. Values for AAV titer (vector genomes/mL and capsids/mL), levels of packaged host-cell DNA (HCD) and plasmid DNA impurities, percent full capsids, and potency—all key CQAs mainly defined by plasmid design and upstream process parameters—were determined.

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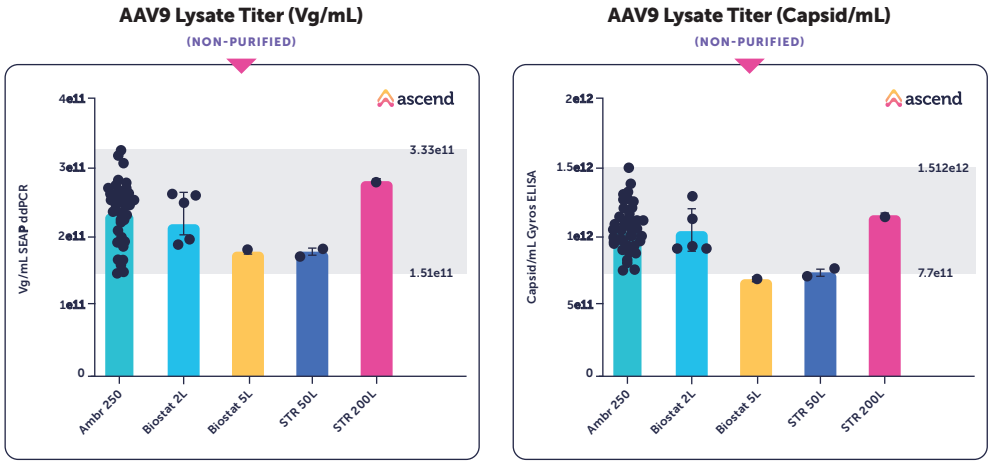
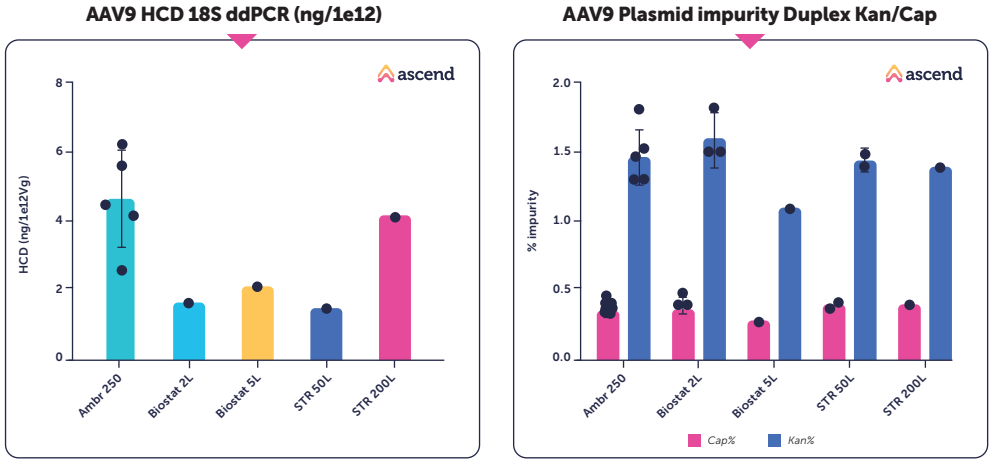
Figure 1: Consistent non-purified lysate titers



Non-purified lysate titer values are presented in **Figure 1**. The vector genome (VG) yields were analyzed using a droplet digital polymerase chain reaction (ddPCR) assay. The maximum variation around the average value was found to be 32%, and the highest yield was observed at the 200L scale. Capsid yields were determined using an enzyme-linked immunosorbent assay (ELISA) kit from Gyros Protein Technologies. The variation in these values was even less than that for the VG titer.

Data for HCD and total plasmid DNA (kanamycin and capsid gene) impurity packaging levels for vector production at different scales are shown in **Figure 2**. Analysis was performed on samples collected after purification. The levels of both types of impurities fell at the low end of the ranges reported in the industry for AAV vectors (approx. 20 ng/1e12 VG and 5-10%, respectively) for all process scales.

Figure 2: Low levels of DNA impurity packaging



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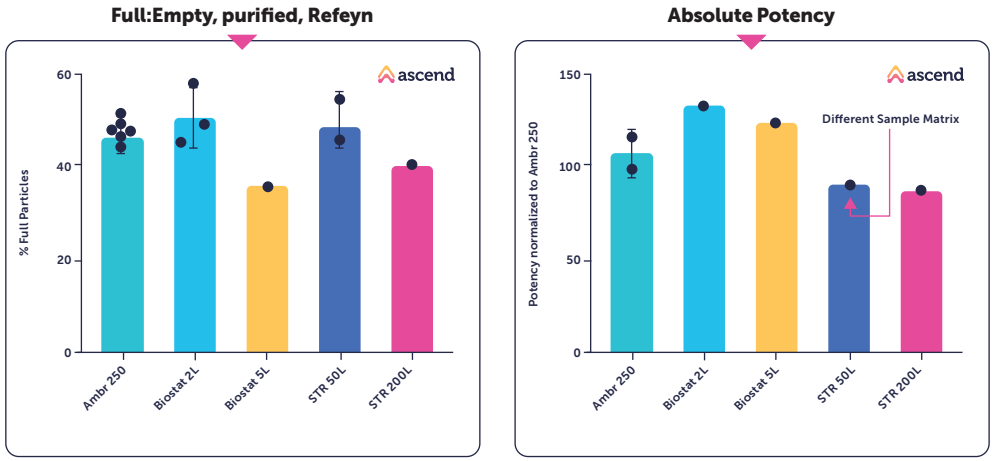
The presence of empty capsids in AAV vectors intended for use as gene therapies has come under increasing scrutiny due to rising concerns about their impact on safety and efficacy. The percent full capsids, or full/empty ratio, is therefore an important CQA. The Ascend platform process using EpyQ was designed to deliver a high percentage of full capsids even without performing a specific chromatographic purification step to remove empty capsids.

Figure 3: High percent full AAV capsids with comparable potency



Importantly, this ability to produce a high percentage of full capsids is retained across scales. As can be seen in **Figure 3** (left-hand side), after purification (not including any steps to remove empty capsids) a high percentage of full capsids (37% to 55%) was observed for the AAV9-SEAP vector runs performed from 250 mL to 200L as determined via Refeyn® Two mass photometry analysis. The observed small variation may be attributed to analytical method variance or differences in the purification protocol (quick-spin purification for small scale, one-step affinity chromatography at medium scale, and full downstream purification for the 50 and 200L scale runs. Given the similar high percentages of full capsids obtained for the different runs, it is not surprising that the vectors produced at different scales exhibited comparable potency (**Figure 3**, right-hand side).

Expedite projects with tailored solutions

Production of the AAV9-SEAP vector in the above study was achieved using the EpyQ AAV production system. It is important to note, however, that the platform also supports scalable AAV vector production with a similar balance of yield and quality via the conventional triple transfection processes.

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Figure 4: Direct scale up of client processes from lab (Ambr 250 mL) to final scale

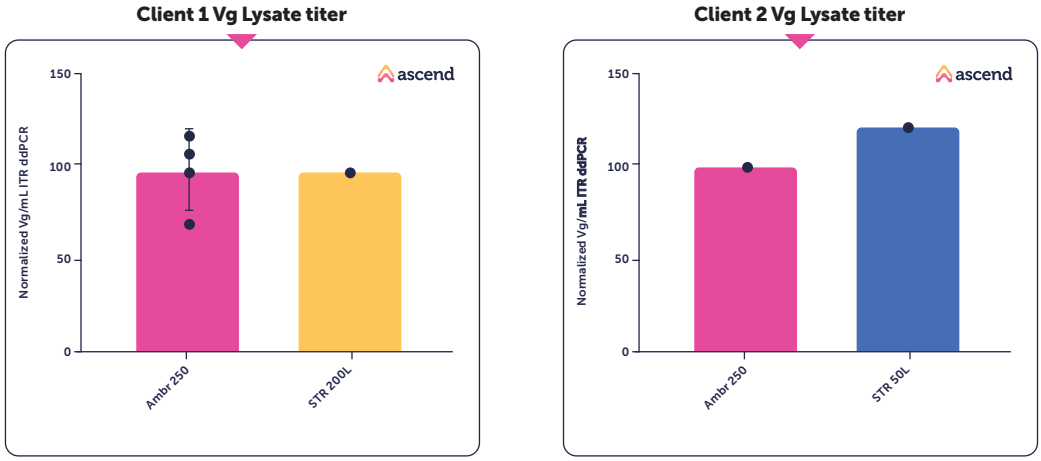

Figure 4 presents the results for two different client processes when directly scaled from the Ambr 250 system to the desired final scale (either 50L or 200L) using the client's triple plasmid system. Despite this customization, the viral titer of the non-purified lysate obtained at the higher scale was in one case nearly the same as that obtained at lab scale, while in the other it was measurably higher.

Designing quality in from the beginning

Ascend has designed a Gene to GMP offering in the United States and Europe that supports a wide spectrum of AAV-based gene therapies. Working with partners like Sartorius, we work to reduce development timelines, increase quality and safety and ultimately break down accessibility barriers for patients. As demonstrated above, working with a flexible and consistent upstream manufacturing platform that scales from the bench-scale to 200L production scale while still meeting stringent quality targets is one way to move closer to these goals.

Please reach out any time at business@ascend-adv.com and keep up with our latest updates on LinkedIn.

To learn more about Sartorius bioreactors, please visit:
https://www.sartorius.com/en/products/fermentation-bioreactors